SCREENING OF EXPRESSION PROFILE OF MUSCLE SPECIFIC GENES EXPRESSED BY GROWING STAGES IN SWINE AND FUNCTIONAL CDNA CHIP PREPARED BY USING THE SAME

Technical Field

The present invention relates to screening of expression profile of muscle specific genes according to growing stages of swine and a functional cDNA chip using the same. More particularly, the present invention relates to screening of expression profile of muscle specific genes specifically expressed in the muscle and fat tissues of swine according to the growing stages and a functional cDNA chip for evaluating high meat quality and screening of specific genes of swine prepared by integrating only the muscle specific genes.

Background Art

Since native black swine has a thick back fat layer and shows a low growth rate and a low production rate, the pig farmers do not prefer to raise it. However, this swine has solid fat tissue, white fat color, excellent texture, abundant and sweet gravy, which suits our taste and thus, its consumption is recently tending to increase. However, genetic research of the native swine, preservation and control of pedigree, analysis of meat quality related genes are still insufficient. Particularly, the meat quality related genetic traits are composite results of more genetic traits, as compared to the meat quantity related traits and research on this has not been much conducted (Cameron, 1993).

Important genes affecting meat quality in swine which have been known to so far include ryanodine receptor gene (RYR) resulting in PSE (pale, soft, exudative) pork meat (Eikelenboom and Minkema, 1974; Smith and Bampton, 1977; Webb, 1981; Christian and Mabry, 1989; Fujii el al., 1991) and acid meat genes (Rendement Napole, Le Roy el al., 1990; Lundstrom el al., 1996). In addition, by QTL (quantitative trait loci) analysis, meat quality related regions or various candidate genes are known. Swine leucocyte antigen (SLA) composite existing in No. 7 chromosome (Geffrotin el al., 1984) and micorsatellite marker S0064, S0066, S0102 or TNF around this region are known to be associated with back fat thickness, sirloin unit area, meat quality traits, boar taint (Jung el al., 1989; Rothschild el al., 1995; Bidanel el al., 1996). Also, it has been found that back fat thickness- and abdominal fat content-related QTL is present in positions of microsatellite marker S0001 to S0175 (Andersson el al., 1994). Further, it has been reported that the pituitary-specific transcription factor (PIT1) gene which is known as a regulation factor of hormones (Yu el al., 1995). The intramuscular fat content (IMF) is known to largely affect the tenderness, juiciness and taste of meat (Devol el al., 1988; Cameron, 1990). H-FAPB (heart-fatty acid binding protein) has been reported as a gene which exerts influence on the intramuscular fat content (Gerbens el al., 1997). The Microsatellite SW1823 to S0003 (74 to 79cM) positions existing in No. 6 chromosome has been studied on the relation of such properties of meat (Grindflek el al., 2001).

Thus, as QTL affecting meat quality traits was largely found in NO. 4, 6 and 7 chromosomes (Clamp el al., 1992; Andersson el al., 1994; Renard el al., 1996; Rohrer and Keele 1998a, 1998b; Wang el

al., 1998; de Koning el al., 1999; Ovilo el al., 2000; Gerbens el al., 2000), much research has been conducted to develop a meat quality related marker centering around these chromosome.

For last few years, there have been efforts to develop a gene map comprising anonymous meat quality-related gene markers of swine and known markers. Up to now, several technologies to analyze gene expression at the mRNA level such as northern blotting, differential display, sequential analysis of gene expression and dot blot analysis have been used to examine the genetic difference in swine. However, these methods have disadvantages which are not suitable for simultaneous analysis of a plurality of expressed products. In recent, a new technology such as cDNA microarray to overcome such disadvantages has been developed. The cDNA microarray becomes one of the strongest means to study gene expression in various living bodies. This technology is applied to simultaneous expression of numerous genes and discovery of genes in a large scale, as well as polymorphism screening and mapping of genetic DNA clone. It is a highly advanced RNA expression analysis technology to quantitatively analyze RNA transcribed from already know or not-known genes.

DNA chip types which are currently used include composite DNA chips constructed by designing a primer based and combining genes from cDNA library on the data base information and functional DNA chips constructed by combining related genes based on the existing references. When the composite DNA chip is used for translation, there is difficulty in translation due to the action of non-related genes and enormous efforts are required to finally interpret the biological roles. Also, since it is based on the database, there may be difficulties due to a new gene without information or

possibility of partial absence of related gene. Meanwhile, the functional DNA chip is easy to be translated but requires another collection of genes for characterization of genes which are not described in the existing references or not-know for their functions. Therefore, the DNA construction on a chip is very important for effective interpretation.

Considering these matters, the present inventors have introduced the cDNA microarray technology into screening of the expression profile of genes related to meat quality in a specific tissue of swine and made a functional cDNA chip by integrating only the specific gene identified from the screening which would be applied to swine improvement with high meat quality and evaluation of meat quality according to breeds and tissues of swine.

Disclosure of Invention

Therefore, an object of the present invention is to screen an expression profile of specific genes differentially expressed according to growing stages of the muscle by hybridizing a substrate integrated with a probe prepared from total RNA isolated from the muscle and fat tissues of swine with a target DNA from the muscle and fat tissues of swine.

It is another object of the present invention to provide a functional cDNA chip for meat quality evaluation and screening of specific genes in swine, which is prepared by integrating only the specific genes obtained from the screening.

According to the present invention, the above-described objects are accomplished by preparing thousands of ESTs from total RNA isolated from the muscle and fat tissues of swine by PCR,

cloning them to analyze and screen their nucleotide sequences in the database, amplifying the ESTs by PCR, followed isolation and purification, arraying the product with a control group on a slide using a DNA chip array, preparing a target DNA from total RNA isolated from the muscle and fat tissues of swine to screen an expression profile of a growth-related gene, hybridizing the slide (probe DNA) with the target DNA, scanning the product to obtain an image file, examining the expression aspect of the muscle-related gene differentially expressed according to the growing stages of swine based on the image file, and preparing a functional cDNA chip by integrating only the muscle specific genes of swine according to the growing stages.

The present invention comprises the steps of preparation of ESTs from muscle and fat tissues of swine and identification of sequence information; preparation of a probe DNA using the ESTs; hybridization of a fluorescent-labeled target DNA (ESTs) from the muscle and fat tissues of swine with the probe DNA, followed by scanning and analysis of an image file; examination of the expression profile of a muscle-related genes according to growing stages in swine; and preparing a functional cDNA by integrating only the muscle specific gene.

The functional cDNA chip for meat quality evaluation and screening of specific genes in swine is prepared by the following steps: preparing 4434 ESTs from total RNA isolated from the muscle and fat tissues of swine by PCR; arraying the ESTs with an enzyme control on a slide using a DNA chip array; preparing a target DNA having 3-dCTP or 5-dCTP bound from total RNA isolated from the muscle and fat tissues of swine; hybridizing the slide (probe DNA)

with the target DNA, scanning the product and analyzing the image file to examine the expression aspect of the muscle-related genes specifically expressed according to the growing stages in swine; and preparing a functional cDNA chip by integrating only the screened muscle specific gene according to the growing stages in swine.

The functional cDNA chip for meat quality evaluation and screening of specific genes in swine according to the present invention comprises a probe comprising muscle specific genes specifically expressed in the muscle and fat tissues of swine and a substrate on which the probe is immobilized.

The probe DNA immobilized on a DNA microarray of the functional cDNA chip for meat quality evaluation and screening of specific genes in swine according to the present invention includes ESM-specific genes and ASM-specific genes.

The ESM-specific gene immobilized on a DNA microarray of the functional cDNA chip for meat quality evaluation and screening of specific genes in swine according to the present invention include actin, beta-myosin, glycogen phosphorylase, myosin heavy chain, pyruvate kinase and troponin C coding gene.

The ASM-specific gene immobilized on a DNA microarray of the functional cDNA chip for meat quality evaluation and screening of specific genes in swine according to the present invention include 1-alpha dynein heavy chain, 601446467F1, fibronectin precursor and MHC class I coding gene.

The substrate of the functional cDNA chip according to the present invention is preferably a polymer film such as silicone wafer, glass, polycarbonate, membrane, polystyrene or polyurethane. The DNA microarray according to the present invention may be

prepared by immobilizing a probe on a substrate by a conventional method for preparing a DNA microarray, including photolithography, piezoelectric printing, micro pipetting, spotting and the like. In the present invention, the spotting method is used.

The kit for meat quality evaluation and screening of specific genes in swine comprises the functional cDNA chip having the muscle specific genes according to the growing stages in swine integrated, Cy5-dCTP or Cy3-dCTP bound cDNA from RNA of the tissue to be screened, a fluorescence scanning system and computer analysis system.

Best Mode for Carrying Out the Invention

Now, the concrete construction of the present invention will be explained through the following Examples. However, the present invention is not limited thereto.

[Example]

Example 1: Screening of expression profile of muscle specific genes according to the growing stages in swine

In order to screen the expression profile of muscle specific genes specifically expressed according to the growing stages in swine, a probe DNA was prepared from total RNA isolated from muscle and fat tissues of Kagoshima Berkshire and the total RNA of the tissues was fluorescently labeled to prepare a target DNA. These DNAs are hybridized and scanned. The resulting image file was analyzed to screen the muscle specific genes according to the growing stages in swine.

<u>Preparation Example 1: Preparation and array of probe DNA</u>

Firstly, probe DNA, which was cDNA amplified by PCR, was prepared and attached to a slide glass. Total RNA was extracted from the muscle and fat tissues of the longissimus dorsi of *Kagoshima Berkshire* (body weight of 30 kg and 90 kg) using a RNA extraction kit (Qiagen, Germany) according to the manual and mRNA was extracted using an oligo (dT) column. The extracted mRNA sample was subjected to RT-PCR using SP6, T3 forward primer, T7 reverse primer (Amersham Pharmacia Biotech, England) to synthesize cDNA. The total volume of each PCR reactant was 100 $\mu\ell$. 100 pM of forward primer and reverse primer were each transferred to a 96-well PCR plate (Genetics, England). Each well contained 2.5 mM dNTP, 10×PCR buffer, 25 mM $MgCl_2$, 0.2 μg of DNA template, 2.5 units of Taq polymerase. PCR was performed in GeneAmp PCR system 5700 (AB Applied BioSystem, Canada) under the following conditions: total 30 cycles of 30 seconds at 94℃, 45 seconds at 58℃, 1 minute at 72℃.

The size of the amplified DNA was identified by agarose gel electrophoresis. The PCR product was precipitated with ethanol in 96-well plate, dried and stored at -20℃

Total 4434 cDNAs (ESTs), prepared as described above, were cloned to analyze nucleotide sequences of genes which swine has and their genetic information was identified from the database at NCBI. The genes having information were isolated and purified by PCR. The enetic locus and map for the total 4434 cDNAs (ESTs) were constructed. The total 4434 cDNAs (ESTs) and 300 yeast controls were arrayed in an area of 1.7 cm^2. Then, the probe DNA was spotted on a slide glass for microscope (produced by Corning), coated with CMT-GAPSTM aminosilane using Microgrid II (Biorobotics). The probe DNA was printed onto Microgrid II using a split pin. The pin

apparatus was approached to the well in the microplate to inject the solution into the slide glass (1 to 2 nL). After printing of the probe DNA, the slide was dried and the spotted DNA and the slide were UV cross-linked at 90 mJ using Stratalinker TM (Stratagene, USA), washed twice with 0.2% SDS at room temperature for 2 minutes and washed once with third distilled water at room temperature for 2 minutes. After washing, the slide was dipped in a water tank at 95℃ for 2 minutes and was blocked for 15 minutes by adding a blocking solution (a mixture of 1.0 g $NaBH_4$ dissolved in 300 mL of pH7.4 phosphate buffer and 100 mL of anhydrous ethanol). Then, the slide was washed three times with 0.2% SDS at room temperature for 1 minute and once with third distilled water at room temperature for 2 minutes and dried in the air.

<u>Preparation Example 2: Preparation of target DNA and hybridization</u>

In order to prepare a target DNA to screen the muscle specific genes specifically expressed in the muscle and fat tissues of swine, the muscle tissue on the longissimus dorsi area was taken from the *Kagoshima Berkshires* having body weights of 30 kg and 90 kg. The fat tissue was taken from the Kagoshima Berkshire having a body weight of 30 kg. The muscle and fat tissues were cut into 5~8 mm length, frozen with liquid nitrogen and stored at -70℃.

Total RNAs were isolated from 0.2 to 1.0 g of the experimental group and the control group according to the manual of Trizol ™ kit (Life Technologies, Inc.) to prepare the target DNA. Trizol™ was added to the tissue in an amount of 1 mL of Trizol™ per 50 to 100 mg of tissue and disrupted using a glass-Teflon or

Polytron homogenizer. The disrupted granules were centrifuged at 4℃ at a speed of 12,000 g for 10 minutes and 1 mL of the supernatant was aliquoted. 200 μℓ of chloroform was added to each aliquot, voltexed for 15 seconds, placed on ice for 15 minutes and centrifuged at 4℃ at a speed of 12,000 g for 10 minutes. Chloroform of the same amount was again added thereto, voltexed for 15 seconds, placed on ice for 15 minutes and centrifuged at 4℃ at a speed of 12,000 g for 10 minutes. The supernatant was transferred to a new tube. 500 μℓ of isopropanol was added to the tube, voltexed and placed on ice for 15 minutes. The ice was cooled and centrifuged at 4℃ at a speed of 12,000 g for 5 minutes. The supernatant was removed, mixed with 1 mL of 75% cold ethanol and centrifuged at 4℃ at a speed of 12,000 g for 5 minutes. The supernatant was removed, freeze-dried on a clean bench for 30 minutes and take into 20 μℓ of RNase-free water or DEPC water to dissolve RNA. The total DNA concentration was set to 40 μg/17 μℓ for electrophoresis.

The target DNA was prepared according to the standard first-strand cDNA synthesis. Briefly, according to the method described by Schuler (1996), 40 μg of total RNA and oligo dT-18mer primer (Invitrogen Life Technologies) were mixed, heated at 65℃ for 10 minutes and cooled at 4℃ for 5 minutes. Then, 1 μℓ of a mixture of 25 mM dATP, dGTP and dTTP, 1 μℓ of 1 mM dCTP (Promega) and 2 μℓ of 1 mM cyanine 3-dCTP or 2 μℓ of 1 mM cyanine 5-dCTP, 20 units of RNase inhibitor (Invitrogen Life Technology), 100 units of M-MLV RTase, 2 μℓ of 10 × first strand buffer were added thereto and mixed with a pipette. The reaction mixture was incubated at 38℃ for 2 hours and the non-bound nucleotide was removed by ethanol precipitation. Here, DEPC treated sterile water was used.

The slide, prepared above, was pre-hybridized with a hybridization solution (5×SSC, 0.2% SDS, 1 mg/mL herring sperm DNA) at 65℃ for 1 hour. The target DNA labeled with cyanine 3 (Cy-3) and cyanine 5 (Cy-5) was re-suspended in 20 μl of the hybridization solution at 95℃ and denatured for 2 minutes. Then, the slide were hybridized with the solution at 65℃ overnight. The hybridization was performed in a humidity chamber covered with a cover glass (Grace Bio-Lab).

After hybridization, the slide was washed 4 times with 2×SSC, 0.1% SDS at room temperature for 5 minutes while vigorously stirred in a dancing shaker. Then the slide was washed twice with 0.2×SSC for 5 minutes and 0.1×SSC for 5 minutes at room temperature.

The slid was scanned on ScanArray 5000(GSI Lumonics Version 3.1) with a pixel size of 50 μm. The target DNA labeled by cyanine 3-dCTP was scanned at 565 nm and the target DNA labeled by cyanine 5-dCTP was scanned at 670 nm. Two fluorescence intensities were standardized by linear scanning of cyanine 3-dCTP- and cyanine 5-dCTP-labeled spots. The slide was again scanned on Scanarray 4000XL with a pixel size of 10 μm. The resulting TIFF image files were analyzed on Quantarray software version 2.1 and the background was automatically subtracted. The intensity of each spot was put into Microsoft Excel from Quantarray. The results are shown in Table 1 and Table 2.

The entire gene expression pattern of ESM (early stage muscle) was compared with those of ASM (adult stage muscle) and ESF (early stage fat). The "ESM-specific" and "ASM-specific" genes are shown in Table 1 and the "ESF-specific" genes are shown in Table 2. 20 genes showed a 5 times higher expression level in ASM, as

compared to ESM. Also, 18 genes showed a 10 times higher expression level in ESF, as compared to ESM, and a 5 to 10 times higher expression level in ESM, as compared to ASM.

Some of the ASM-specific genes, ESM-specific genes, ESF-specific genes including expected gene groups are shown in Table 1 and Table 2.

【Table 1】

Expression ratio of differentially expressed genes between ESM and ASM

ESTs No.	Accession No. †	Description**	Ratio of gene expression ESM(30) / ASM(90)
Cellular structure and motility			
SM2149	CAB56598	1-alpha dynein heavy chain	-2.1
SM781	NP_033891	19 kDa-interacting protein 3-like	+2.1
SM635	BAB19361	Actin	+3.4
SM713	AAA51586	Actin	+6.3
SM106	P53506	Actin	+8.8
SM1068	AAF20165	Actin	+5.3
SM363	B25819	Actin	+4.3
SM768	X52815	Actin	+3.4
SMk77	NM_001100	Actin, alpha 1	+15.1
SM128	NP_033740	Actin, gamma 2	+6.9
SM902	BC001748	Annexin A2	-3.2
SM846	P81287	Annexin V	-2.8
SM653	P04272	Annexin Ⅱ	-2.2
SMk340	U75316	Beta-myosin heavy chain mRNA	+3.0
SM1605	AAF99682	Calpain large polypeptide L2	+4.7
SM541	NP_000079	Collagen	-3.2
SM715	L47641	Collagen	-6.8
SM430	Q9XSJ7	Collagen alpha 1	-6.8
SM758	CGHU1S	Collagen alpha 1	-2.1
SM62	CGHU2V	Collagen alpha 2	-3.2
SM949	O46392	Collagen alpha 2	-3.3
SM410	CAA28454	Collagen (alpha V)	-2.3
SM1651	XM_039583	Discs, large (Drosophila) homolog 5	-2.0
SM1050	AAA30521	Fibronectin	-2.4
SM491	NM_005529	Heparan sulfate proteoglycan 2	-2.2
SM1573	XM_044160	Lamin A/C	+2.6
SMk55	NP_006462	Myosin	+3.9
SMk338	P79293	Myosin heavy chain	+2.0

SMk168	AB025261	Myosin heavy chain	+9.0
SM1732	NP_004678	Myotubularin related protein 4	+3.8
SM1691	NP_000908	Procollagen-proline	-2.3
SM690	NP_003109	Secreted protein, acidic	-4.4
SMk173	X66274	Tropomyosin	+2.6
SM141	CAA38179	Tropomyosin	+2.7
SMk51	P18342	Tropomyosin alpha chain	+9.6
SM1043	P06469	Tropomyosin alpha chain	+11.5
SMk19	P02587	Troponin C	+14.5
SMk50	Y00760	Troponin-C	+19.6
SMk57	AAA91854	Troponin-C	+14.6
SM1535	P02554	Tubulin beta chain	+2.8
SM1063	P20152	Vimentin	-5.4
Metabolism			
SMk56	AAA37210	Aldolase A	+5.5
SM995	CAA59331	Carbonate dehydratase	+3.2
SMk344	NM_012839	Cytochrome C	+3.4
SM800	AAG53955	Cytochrome c oxidase subunit I	+3.0
SM51	T10974	Cytochrome-c oxidase	+3.8
SMk151	CAA06313	Fructose-1,6-bisphosphatase	+7.1
SM2070	P00339	L-lactate dehydrogenase M chain	+12.7
SMk120	AJ275968	LIM domains 1 protein	+8.6
SMk147	X59418	NADH dehydrogenase	+2.4
SM928	O79874	NADH-ubiquinone oxidoreductase chain 1	+5.3
SMk18	AAG28185	NADH4L	+2.1
SMk81	O19094	Octanoyltransferase(COT)	+3.2
SM295	AB006852	Phosphoarginine phosphatase	+2.6
SMk346	M97664	Phosphoglucomutase isoform 2 mRNA	+5.5
SM36	TVMVRR	Protein-tyrosine kinase	+4.3
SM887	P11980	Pyruvate kinase	+8.5
SM698	S64635	Pyruvate kinase	+9.7
SM723	P52480	Pyruvate kinase	+7.3
SMk79	U44751	Pyruvate kinase	+5.2
SMk135	Z98820	Sarcolipin	+3.0
SM1033	XM_018138	Tyrosine phosphatase type IVA	+2.9
SMk347	X99312	UDP glucose pyrophosphorylase	+3.0
Gene/protein expression			
SM75	U09823	Elongation factor 1 alpha	-4.3
SM1989	AAH05660	Elongation factor 1 alpha 1	-3.9
SMk61	NP_031959	Enolase 3	+3.6
SM968	Y00104	Repetitive dna sequence element RPE-1	-2.5
SMk91	AAC48501	Reticulum protein	+4.6
SM2083	NP_003083	Ribonucleoprotein polypeptide B	+3.1
SM896	AAH01127	Ribosomal protein	+2.0

SM1668	AAH07512	Ribosomal protein L18a	+2.1
SM1784	228176	Ribosomal protein P0	+6.2
SM1801	AAA30799	Transfer RNA-Trp synthetase	+6.0
SM997	51077272	Translation initiation factor eif1	+3.5
Cell signaling / communication			
SM464	AJ002189	Complete mitochondrial DNA	+3.9
SM732	AF304203	Mitochondrion	+5.9
SMk11	XM_006515	Potassium channel	-2.4
SMk187	BC007462	Similar to creatine kinase	+3.5
Cell division			
SM1067	XP_007399	Protease, cysteine, 1	+3.1
Immune response			
SM154	AF036005	Interleukin-2 receptor alpha chain	-2.5
SMk1	AAAG52886	Kel-like protein	+6.4
SM401	AJ251829	MHC class I SLA genomic region	-3.0
EST			
SM824	AK023385	cDNA FLJ13323 fis	+2.5
SM1776	XM_050494	KIAA0182 protein	+3.6
SM1556	XP_043678	KIAA1096 protein	+4.9
Unknown			
SM1785	AC015998	AC015998	+2.1
SM2152	BI327422	AR078G01iTHYEG01S	-4.0
SM1469	BG938561	Cn26h08.x1	-2.2
SM908	AAG28205	COI	+2.8
SM851	AAG28192	COI	+3.6
SM1738	CAA19420	DJ466P17.1.1(Laforin)	+4.8
SM1007	AAD31021	Foocen-m	+3.8
SM1920	BE421626	HWM012cA.1	+3.3
SM1972	XP_039195	Hypothetical protein	+3.2
SM1536	T08758	Hypothetical protein	+4.7
SMk137	XP_002275	Hypothetical protein	+20.0
SM1724	XP_016035	Hypothetical protein	-2.6
SM1539	AT001097	Mandarina library	-2.3
SM1474	BG384994	MARC 1PI	+2.6
SM1853	BF198401	MARC 2PIG	+3.6
SM1941	BE925069	MR1-AN0039-290800-004-a01	+4.4
SM379	AW328623	NIH_MGC_4	+2.3
SM1911	BE872239	NIH_MGC_65	-2.4
SM1676	BG548727	NIH_MGC_77	+5.1
SM1914	BG534187	NIH_MGC_77	-2.3
SM1650	BI337009	Peripheral Blood Cell cDNA library	+9.3
SM1064	BAB28119	Putative	+3.4
SM618	BAB28422	Putative	+2.1
SM1774	BAB30715	Putative	+3.2
SM1690	BF864360	Reinhardtii CC-1690	+2.2
SM1898	F23148	Small intestine cDNA library	-2.3
SM96	M17733	Thymosin beta-4 mRNA	-4.2
SM1922	AAH03026	Unknown	+4.0
SM210	BAA91923	Unnamed protein product	-3.1
No match			
SM107		No match	-2.4
SM278		No match	-2.2
SM384		No match	-2.3

SMk37	No match	+7.7
SM717	No match	-3.0
SM1598	No match	+4.5
SMk6	No match	+3.8
SMk68	No match	+5.0
SM1100	No match	-2.6
SMk70	No match	+3.9
SMk80	No match	+17.7
SMk112	No match	+3.5
SM1639	No match	-4.0
SMk148	No match	+3.8
SM1665	No match	+3.8
SM1665	No match	+13.0
SMk95	No match	+2.7
SMk133	No match	+2.4
SMk152	No match	+6.4
SM1897	No match	+3.4
SMk138	No match	+10.3
SM1902	No match	+2.1
SMk342	No match	+6.7
SMk181	No match	+11.0
SM904	No match	-3.4
SMk262	No match	+3.9
SM9	No match	+2.4
SM1964	No match	+2.6
SMk335	No match	-3.9

† : agreed Accession no.

**: Information agreed to the database

No match: No information agreed to the database; novel EST

ESM: early stage muscle (body weight 30 kg), ASM: adult stage muscle (body weight 90 kg), SM: swine muscle

As shown in Table 1, 14 genes which are expressed in ASM, identified in Table 1 and known for their functions have not yet precisely measured. These genes include actin alpha 1, tropomyosin alpha chain, aldolase A, fructose-1,6-bisphosphatase, NADH-ubiquinone oxidoreductase chain 1, phosphoglucomutase isoform 1 mRNA, pyruvate kinase, mitochondrion, kel-like proteins (Table 2). Actin cytoskeleton comprising microfilaments is responsible for various functions in eukaryotic cells including intracellular transport and structure support. Actin exists in the form of a monomer (G-actin) or filament (F-actin). The F-actin is a main component of the

microfilament. Many proteins regulate the length, location and transform of the microfilament. The actin cytoskeleton has a variable structure which can immediately change the shape and structure in response to a stimulus and in the course of the cell cycle. The structure of the actin cytoskeleton is not fixed but varied in response to the cellular environment. Tropomyosin with troponin complexes (troponin-I, -T and C) bonded thereto plays an important role in Ca^{2+} dependent regulation upon contraction of linear muscle in vertebrata. Tropomyosin is closely connected to a protein group having an alpha coiled coil structure comprising a dimmer. Pyruvate kinase which catalyzes transphosphorylation of PEP to ADP in mammals is one of the important regulation enzymes and its property to regulate the metabolic pathways is closely involved in various metabolic demands needed in other tissues during pathway regulation. Thus, the present inventors use it as an object of study.

Also, 5 genes which are expressed in ESM, identified in Table 1 and Table 2 and not known for their functions have not yet precisely measured. These genes include collagen, disk/large homologue 5 (fruit fly), acid secret proteins, vimentin. Collagen is a main component of extracellular matrix and comprises at least 18 types of different macro protein groups, which are observed upon cell division, replication, migration and attachment in the course of embryo development and various morphological differentiations and partially regulated by the cellular interaction of surrounding extracellular matrix.

The expression of vimentin coding genes (Vim) is one of the terminal markers which appear after a serial of genetic events

occurring in the course of differentiation of leukocyte to macrophage. Therefore, valuation of transcriptional regulation mechanism is an important stage to understand the genetic regulation pathways responsible for the leukocyte differentiation.

【Table 2】

Expression ratio of differentially expressed genes between ESM and ESF

ESTs No.	Accession No†.	Description**	Ratio of gene expression ESF(30) / ESM(30)
Cellular structure and motility			
SM2149	CAB56598	1-alpha dynein heavy chain	-2.1
SM781	NP_033891	19 kDa-interacting protein 3-like	+2.2
SM1068	AAF20165	Actin	+4.5
SM635	BAB19361	Actin	+2.6
SM106	P53506	Actin	+4.9
SM768	X52815	Actin	+2.4
SM363	B25819	Actin	+3.7
SM713	AAA51586	Actin	+5.6
SMk77	NM_001100	Actin, alpha 1	+4.5
SM128	NP_033740	Actin, gamma 2	+3.9
SM1091	JC5971	Alpha-b crystallin	+2.1
SM902	BC001748	Annexin A2	-4.2
SM846	P81287	Annexin V	-3.5
SM653	P04272	Annexin II	-2.3
SMk340	U75316	Beta-myosin heavy chain mRNA	+2.2
SM1807	AAF99682	Calpain large polypeptide L2	+2.7
SM541	NP_000079	Collagen	-4.9
SM715	L47641	Collagen	-5.2
SM1023	Q9XSJ7	Collagen alpha 1	-4.6
SM758	CGHU1S	Collagen alpha 1	-4.3
SM62	CGHU2V	Collagen alpha 2	-4.4
SM949	O46392	Collagen alpha 2	-3.2
SM410	CAA28454	Collagen(alpha V)	-2.3
SM1121	NM_000393	Collagen, type V, alpha 2	-2.8
SM53	NP_000384	Collagen, type V, alpha 2	-2.5
SM1651	XM_039583	Discs, large(Drosophila) homolog 5	-8.6
SM1050	AAA30521	Fibronectin	-3.1
SM381	FNHU	Fibronectin precursor	-2.6
SM122	P07589	Fibronectin(FN)	-2.5
SM1573	XM_044160	Lamin A/C	+2.1
SMk55	NP_006462	Myosin	+3.6
SMk168	AB025261	Myosin heavy chain	+5.0
SM1732	NP_004678	Myotubularin related protein 4	+4.7
SM690	NP_003109	Secreted protein, acidic	-5.2

SM1043	P06469	Tropomyosin alpha chain	+8.6
SMk173	X66274	Tropomysin	+2.2
SMk19	P02587	Troponin C	+6.9
SMk57	AAA91854	Troponin-C	+7.1
SMk50	Y00760	Troponin-C	+9.0
SM1535	P02554	Tubulin beta chain	+3.3
SM1063	P20152	Vimentin	-5.1
SM730	CAA69019	Vimentin	-3.2
Metabolism			
SMk344	NM_012839	Cytochrome C	+2.4
SM800	AAG53955	Cytochrome c oxidase subunit I	+2.9
SMk151	CAA06313	Fructose-1,6-bisphosphatase	+4.2
SMk254	231300	Glycogen Phosphorylase b	+2.6
SM2070	P00339	L-lactate dehydrogenase M chain	+10.6
SM928	O79874	NADH-ubiquinone oxidoreductase chain 1	+3.2
SMk81	O19094	Octanoyltransferase(COT)	+3.9
SM295	AB006852	Phosphoarginine phosphatase	+2.3
SMk346	M97664	Phosphoglucomutase isoform 2 mRNA	+3.3
SM36	TVMVRR	Protein-tyrosine kinase	+2.6
SM723	P52480	Pyruvate kinase	+7.5
SM698	S64635	Pyruvate kinase	+6.6
SM887	P11980	Pyruvate kinase	+6.3
SM1594	AAA62278	Superoxide dismutase	-3.2
SM1033	XM_018138	Tyrosine phosphatase type IVA	+2.2
Gene/protein expression			
SM75	U09823	Elongation factor 1 alpha	-3.7
SM1989	AAH05660	Elongation factor 1 alpha 1	-3.8
SMk120	AJ275968	LIM domains 1 protein	+9.9
SMk91	AAC48501	Reticulum protein	+2.1
SM2083	NP_003083	Ribonucleoprotein polypeptide B	+3.2
SM21	NP_000994	Ribosomal	+2.2
SM1784	228176	Ribosomal protein P0	+5.5
SM1820	BC014277	Tissue inhibitor of metalloproteinase 3	-2.6
SM1801	AAA30799	Transfer RNA-Trp synthetase	+5.7
SM997	51077272	Translation initiation factor eif1	+2.3
Cell signaling / communication			
SM464	AJ002189	Complete mitochondrial DNA	+2.7
Immune response			
SMk1	AAG52886	Kel-like protein 23	+4.6
EST			
SM1776	XM_050494	KIAA0182	+3.2
SM1556	XP_043678	KIAA1096 protein	+4.5
Unknown			
SM2152	BI327422	AR078G01iTHYEG01S	-5.5
SMk3	AL13277	Chromosome 14 DNA sequence	+2.3
SM908	AAG28205	COI	+2.2
SM1738	CAA19420	DJ466P17.1.1(Laforin)	+3.5
SM1007	AAD31021	Foocen-m	+3.0
SM1724	XP_016035	Hypothetical protein	-2.6
SMk137	XP_002275	Hypothetical protein	+10.0
SM1972	XP_039195	Hypothetical protein	+2.8
SM787	AF192528	Integrin beta-1 subunit	+2.0
SM1474	BG384994	MARC 1PI	+2.8

SM1676	BG548727	NIH_MGC_77	+2.3
SM1650	BI337009	Peripheral Blood Cell cDNA library	+7.3
SM1774	BAB30715	Putative	+5.1
SM1064	BAB28119	Putative	+3.0
SM1690	BF864360	Reinhardtii CC-1690	+2.5
SM96	M17733	Thymosin beta-4 mRNA	-3.9
SM1922	AAH03026	Unknown	+4.7
No match			
SMk58		No match	+2.9
SM717		No match	-4.4
SMk6		No match	+2.4
SMk68		No match	+3.2
SMk80		No match	+4.3
SMk112		No match	+2.1
SM1639		No match	-2.8
SMk148		No match	+2.9
SM1665		No match	+9.8
SMk95		No match	+2.1
SMk152		No match	+6.4
SM1897		No match	+2.6
SMk138		No match	+3.1
SM796		No match	-2.2
SMk342		No match	+3.9
SMk181		No match	+4.4
SM904		No match	-2.7
SMk262		No match	+2.7
SM9		No match	+2.9
SM1964		No match	+2.6
SMk335		No match	+3.8

† : agreed Accession no.

**: Information agreed to the database

No match: No information agreed to the database; novel EST

ESM: early stage muscle (body weight 30 kg), ESF: early stage fat (body weight 30 kg), SM: swine muscle

As shown in Table 2, 13 genes include expressed in ESF include troponin -C, L-lactate dehydrogenase M chain, LIM domain 1 protein, pyruvate kinase, ribosome protein P0, transfer RNA-Trp syntase. The genome clones comprising human pyruvate kinase M(PKM) genes encoding M1 type and M2 type isozyme were isolated and measured for their exon sequences. The genes were about 32 kb and comprise 12 exons and 11 introns. The exon 9 and 10 comprise sequences specific to the M1 type and M2 type, respectively, which

indicates that the human isozyme is produced from the same gene by selective splicing, like the genes of rat. 4½LIM domain protein 1(FHL1) was initially used as an abundant skeletal muscle protein having 4 LIM domains and 1 GATA such as zinc finger. FHL1 was shown to be expressed in the skeletal muscle as well as various tissues. In recent, it has been identified that selectively inserted FHL1 mRNA encodes proteins with the C-end deleted. It was found that FHL1C ultimately produces N-end comprising 16 amino acids in the skeletal muscle of sine by a newly identified initiation codon. From the above results, these genes were evaluated as meat quality-related candidate genes.

Thus, the expression rate was 2 times more for genes identified in ESM vs ASM and ESM vs ESF. By cDNA microarray analysis, total 128 genes which had been significantly over-expressed were identified. Actin, beta-myosin, glycogen phosphorylase, myosin heavy chain, novel genes, pyruvate kinase, troponin C were specifically expressed in ESM. collagen, fibronectin, an inhibitor of metalloproteinase 3, intergrin beta-1 sub-unit were specifically expressed in ESF. 1-alpha dynein heavy chain, 601446467F1, assumed protein, fibronectin precursor, MHC class I, novel genes, anonymous protein products were specifically expressed in ASM. These genes were evaluated as meat quality-related candidate genes. Also, the present inventors, from now on, will conduct research on functions of more genes to bring a high meat quality swine.

Example 2: Construction of the inventive functional cDNA chip for meat quality evaluation and screening of specific genes in swine

The muscle specific genes according to the growth stages in swine, screened in Example 1, including the ESM-specific genes such as actin, beta-myosin, glycogen phosphorylase, myosin heavy chain, novel genes, pyruvate kinase and troponin C coding genes and the ASM-specific genes such as 1-alpha dynein heavy chain, 601446467F1, assumed protein, fibronectin precursor and MHC class I coding genes were immobilized on a DNA microarray and fabricated into a functional cDNA chip for meat quality evaluation and screening of specific genes in swine by the method of Preparation Example 1.

Example 3: Construction of the inventive kit for meat quality evaluation and screening of specific genes in swine

A kit for meat quality evaluation and screening of specific genes in swine comprising the functional cDNA chip fabricated in Example 2, Cy5-dCTP or Cy3-dCTP bound cDNA from RNA of the tissue to be screened, a fluorescence scanning system and a computer analysis system was fabricated.

Industrial Applicability

As explained through the Examples, the present invention relates to screening of the expression profile of muscle specific genes according to the growing stages in swine and a functional cDNA chip using the same and provides expression files of the muscle specific genes specifically expressed according to the growing stages in the muscle and fat tissues of swine. Also, the present invention provides a functional cDNA chip for meat quality evaluation and screening of specific genes in swine prepared by integrating only the muscle specific genes screened as described

above. Therefore, the functional cDNA chip can be used to evaluate of meat quality according to breeds of swine and to bring a high meat quality swine, thereby being very useful for the hog raising industry.